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AGAINST Q-FEVER  
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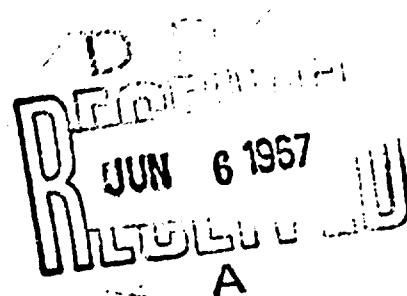
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EXPERIENCE ON THE MASS IMMUNIZATION OF HUMANS WITH THE M-44 LIVE VACCINE  
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Report 1. Subcutaneous Method of Immunization.

[Following is the translation of an article by V. A. Genig, E. N. Knyazeva, P. S. Tselnikov\* and M. M. Miroshnichenko\*\*, N.F. Gamaleya Institute of Epidemiology and Microbiology, published in the Russian-language periodical Voprosy Virusologii (Problems of Virology), No 3, 1965, pages 319--323. It was submitted on 30 Jun 1964. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

Up until the present time the practical application of killed vaccine against Q-fever has been limited in view of its high reactogenicity and the complexity of application [6, 12, 17, 19, 20, 21]. The first attempts at creating improved mildly reactogenic vaccines were not very successful [11, 18]. This circumstance made it necessary to seek weakly pathogenic strains of *Rickettsia burneti* suitable for use as a live vaccine. As a result of numerous years of studying various strains of *R. burneti* cultivated in chick embryos the attenuated variant M-44 of *R. burneti* was developed in the Department for Typhus and other Rickettsioses, headed by P.F. Zdrodovskiy. Based on its properties the M-44 variant turned out to be suitable for use as a live vaccine against Q-fever [1, 2, 4, 9].

In the current work we will present a comparative study of the reactogenicity and immunological effectiveness of the subcutaneous, cutaneous and oral methods of vaccination with the live M-44 vaccine in an extensive experiment on the immunization of humans. The aims were to reveal the most effective and at the same time the most simple method guaranteeing mass immunization. In the present report we will present the results of studying the reactogenicity and immunological effectiveness of the live M-44 vaccine against Q-fever following the subcutaneous application of the vaccine. (75)

Materials and Methods

For the subcutaneous immunization of persons we used 2 series of live vaccine which represented freeze-dried 0.1% suspensions of egg cultures of *R. burneti* from an attenuated M-44 variant, diluted in skimmed sterile milk. The infectious titer of the dry vaccine on chick embryos fluctuated from  $10^{-9}$  --  $10^{-10}$ . During the subcutaneous immunization of guinea pigs the minimum immunizing doses capable of causing the formation of complement fixing antibodies and the resistance of the animals to infection with a virulent culture corresponded to a vaccine dilution of  $10^{-8}$  --  $10^{-9}$ . Standardization of each series of vaccine was carried out on the basis of the data from a determination of the minimum infecting doses of vaccine for chick embryos

\* Chitinskiy Institute of Epidemiology, Microbiology and Hygiene

\*\* Kirghiz Republic Sanitation-Epidemiological Station

(MIDE). On the basis of the results obtained during the study of the immunological effectiveness of various doses of live M-44 vaccine in tests on animals and volunteers, we used an inoculating dose of vaccine corresponding to  $10^5$  --  $10^6$  MIDE for subcutaneous vaccination. In individual cases the inoculating dose equaled  $10^4$  MIDE.

Each ampoule of dry vaccine was diluted in 5 ml of physiological solution, which corresponded to a vaccine dilution of  $10^{-4}$ . The vaccine was administered one time in a volume of 1 ml in the subscapular area. The inoculation reactions were considered on the basis of objective and subjective data. For studying the antigenicity of live vaccine we carried out dynamic observations of 1112 inoculated subjects during the first 5 days following immunization. Later observations were performed by means of questioning the subjects in 10--18 days and by means of serological investigations in 1--4,  $5\frac{1}{2}$  months and one year after vaccination.

For revealing possible late specific reactions we considered and examined serologically subjects in which from the 5th through the 45th day following inoculation febrile diseases in the form of influenza, bronchitis and pneumonia were observed in various periods.

Immunological effectiveness of the vaccine was evaluated based on the presence and expressiveness of specific antibodies, determined in the complement fixation reaction which we set up by the generally accepted method in the cold. The reaction was considered immediately after completion of hemolysis in control test tubes, and then in an hour. The sera from the subjects were investigated in dilutions of 1:5--1:640. All told 1006 sera were investigated in various periods following immunization.

The mass inoculations with live vaccine were carried out in regions unfavorable for Q-fever, where in 1954--1957 and up to the present time Q-fever has been recorded in the form of outbreaks and individual sporadic cases [3, 5, 7, 8, 10, 13--16].

On the basis of epidemiological data and previously conducted serological investigations the mass inoculations against Q-fever were carried out among workers from enterprises processing raw materials and products from livestock breeding, personnel servicing animal raising farms, students from FZU schools (industrial training schools), technical schools and institutes carrying out practical work at meat-packing plants, and workers in rickettsia laboratories.

Inoculations with live vaccine against Q-fever were performed on persons who had and did not have specific antibodies in the blood prior to inoculation. This made it possible to study the expressiveness of allergic reactions during the immunization of persons against a positive sero-immunological background. All told 2020 persons were inoculated. Of these, 4--47% had complement fixing antibodies in the blood prior to immunization. The age of the subjects fluctuated from 15 to 57 years, the majority were personnel from 20--40 years old.

## Results

The results of the observations of the subjects showed that in an absolute majority of persons the subcutaneous administration of live vaccine caused no reactions. Only in 4% of the subjects the administration of vaccine was accompanied by a general reaction which was manifested in the form of a 1-day increase of temperature mainly up to  $37.2-37.3^{\circ}$  and a mild weakness or headache. These symptoms appeared in 48--72 hours.

A temporary disruption in the state of health in the subjects during the manifestation of early inoculation reactions in the majority of cases was not accompanied by a loss of the ability to work. Strong and moderate temperature reactions and a temporary disruption in the ability to work were noted in 1.4% of the subjects. However, these reactions were manifested during the superposing of secondary infections (catarrh of the upper respiratory tract, angina, influenza) or were connected with the appearance of accompanying diseases (rheumatic carditis, expressed thyrotoxicosis, hypertonia, kidney diseases). This was confirmed by the data of clinical-serological investigations.

In a number of cases only a subjective manifestation was noted in the subjects. This was in the form of a temporary chill or a weakly expressed headache not accompanied by an increase in temperature. These symptoms were displayed after 24 hours or in 2--4 days and were noted considerably more often in groups of subjects in which the initial sero-immunological background was high. However, inoculation reactions arising in persons having specific antibodies in the blood prior to inoculation did not differ from the analogous reactions observed in persons not having specific antibodies prior to inoculation. They were expressed weakly and passed rapidly (duration of 4--6 hours). This testifies to the feasibility of carrying out the immunization of persons with live vaccine against Q-fever without a preliminary selection of a threatened contingent based on sero-immunological tests.

The results of the observations on the subjects also showed that the manifestation of early inoculation reactions was varied depending on the age of the subjects and the magnitude of the inoculating dose. Subjects in the age group of 16--18 reacted to the inoculation more weakly than older subjects. The greatest number of temperature reactions (5%) was noted in the 30--40 age group. In the oldest subjects we observed primarily a subjective manifestation of inoculation reactions without an increase in temperature (table 1).

The number of early inoculations reactions with an increase of temperature increased somewhat with an increase in the inoculating doses, and following the administration of vaccine in doses corresponding to  $10^4$  and  $10^5-10^6$  MIDE fluctuated accordingly from 2.9 to 5.3%. However, a direct correlation was not observed between the severity of the early inoculation reactions and the subsequent development of immunity. In the group of subjects with a general reaction to the inoculation and in the subjects without inoculation reactions the number and expressiveness of sero-immunological

reactions in 1--3 months following vaccination were practically the same. However, after average and strong inoculation reactions which arose on a background of superposed secondary infections, the sero-immunological reactions were negative or we observed a very weak development of specific antibodies in titers of 1:5--1:10.

A local reaction was noted in 4.3% of the subjects having specific antibodies in the blood, and in 6.6% of the subjects who did not have them prior to inoculation. This reaction appeared on the 3--4th day in the form of a diffuse swelling, from time to time with a hyperemia of no more than 3--4 cm, and was preserved for 2 dyas. Late specific reactions, analogous to the reactions which appear in remote periods (9--15 days) following the administration of live typhus E vaccine, were not observed during vaccination with the live vaccine against Q-fever.

As a result of the serological investigations, carried out in various periods following immunization, it was established that after a single administration of live vaccine in doses of  $10^5$ -- $10^6$  MIDE the immunological reorganization of the organism set in rapidly.

In the sera of the subjects specific antibodies appeared already in 14--21 days following inoculation and reached the most expressed titers in 2 months in 80% of the subjects. After 3--4 months positive results were preserved in 74.2%, after 5½ months -- in 67%, and after one year -- in 42% of the subjects. The largest number of positive reactions was noted in titers of 1:5 - 1:20, which after 1--4 months made up 42.8--77% of the positive results. Antibody titers of 1:40 - 1:80 made up 19.5--48.2%, and antibody titers of 1:160 - 1:640 -- all told 2.9--9%. The titers of sera, investigated in later periods, in the majority of cases did not exceed 1:5 - 1:20 (table 2). When using inoculation doses corresponding to  $10^4$  MIDE positive reactions were noted after 3 months in 64--77.8% of the subjects. In the majority of cases the antibody titers fluctuated within 1:5 - 1:20. In the persons who had specific antibodies in the blood, after 1--5 months following inoculation positive serological results comprised 84--90.9%. The level of antibody titers increased on an average of two times.

Thus, the results of the serological investigation testify that inoculation doses corresponding to  $10^5$ -- $10^6$  MIDE are effective immunizing doses. With a single administration they ensure a rapid creation and prolonged preservation of immunity.

#### Conclusions

1. During subcutaneous administration in an extensive experiment the live M-44 vaccine against Q-fever turned out to be harmless, mildly reactogenic and immunogenic. This permits us to recommend it for practical application.

2. The absence of expressed allergic reactions following the immunization of persons who had had Q-fever or who reacted positively prior to inoculation testifies to the feasibility of carrying out mass inoculations of threatened contingents without a preliminary sero-immunological selection.

3. Inoculations with live vaccine against Q-fever should be carried out in regions unfavorable for Q-fever and primarily among workers at enterprises engaged in the industrial processing of raw materials and products from the animal raising industry, and among persons working on animal raising farms.

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Reactogenic properties of live M-44 vaccine against Q-fever following subcutaneous administration of the vaccine

Age (in years)	Number inoculated	Number of general reactions (in %)				Percent with loss of work ability
		Total	Temperature increase			
			up to 37.5°	37.5- 38.5°	over 38.5°	
16--18	262	2.6	2.6	--	--	--
20--30	399	4.5	2.8	1.2	0.5	1.5
30--40	335	5.0	2.6	2.1	0.3	2.7
40--50	116	2.6	1.7	0.9	--	0.9
Total	1112	4.0	2.6	1.2	0.2	1.4

Table 2

Immunogenic properties of live M-44 vaccine against Q-fever following subcutaneous administration of the vaccine

Group of subjects	Period of in- vestigation (in months)	Number of in- vestigated sera	Percentage positive	Average anti- body titer	Percentage of positive reactions in titers of			
					1:5 - 1:20	1:40 - 1:80	1:160 - 1:320	1:640
Serum- negative  Persons w/o specific antibodies in blood prior to inoc.	Up to 1	26	84.0	1:20	81.8	18.2	--	--
	1	61	88.5	1:28	74.0	20.4	5.6	--
	1½	321	80.0	1:47	42.8	48.2	9.0	--
	2	295	74.2	1:28	77.6	19.5	2.9	--
	3-4	91	67.0	1:36	83.6	9.9	4.9	1.6
	5½ 12	38	42.1	1:18	81.2	18.8	--	--